STUDIES ON ABNORMAL SPERMATOZOA IV. MORPHOLOGICAL OBSERVATIONS OF ABNORMAL SPERMATOZOA IN VARIOUS PORTIONS OF GENITAL ORGANS OF MALE ALBINO RATS

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It is generally known that the spermatozoa are completed morphologically by the process of metamorphosis in the seminiferous tubules. Walton ('33)[68] reported that this process is not completed before the spermatozoa leave the tubule and pass through the rete testis into the caput epididymidis. Other investigators stated that the spermatozoa undergo maturation during their travel from testes into the ductus epididymides and finally reach the cauda epididymides, where they are normally preserved; that is to say, the spermatozoa take place the morphological and histological changes accompaning with the maturation in the epididymes. Thus, according to the idea of these latter, the epididymes can be regarded as a reservoir for the spermatozoa and also as the place where they undergo completion of maturation. It has been found that when the matured spermatozoa did not discharge by copulation they undergo regressive changes and subsequently liquefaction occur within the cauda epididymides, particularly within the vas deferens.[61][74]

As regards to the marked morphological changes of the spermatozoa during their travel from the caput into the cauda the following observations were made by many investigators.[1][20]–[23][31][45][46][55][56][59] Some protoplasmic drops are found arround the spermatozoa, the drops exist arround the neck of the spermatozoa in both the testis and caput epididymidis, and arround the middle-pieces of them in the corpus and cauda of epididymis, but not in the vas deferens. Several investigators reported about the changes in size,[20][56][57] and in minute construction[17] of the spermatozoa and also about the appearance of abnormal spermatozoa.[57][62]

As to the physiological changes, it has been known that the spermatozoa in

the testes have not usually fertilizing capacities,[36] but they become to increase the capacities gradually during their travel from testes to the epididymes, reaching the maximum in the cauda.[13)35)44)45)54)69)71)-74)] It has also been noted that both the motility and the survival of spermatozoa increase from the testis to the cauda epididymidis where the maximum capacities are attained ; [1)3)5)8)13)18)-20)26)35)44)45)49)51)53)56)65)66)70)71)73)74)] furthermore, they increase the resistance against the temperature,[63] pH[70] or various toxic drugs.[20] It is known that these physiological changes occured with the functional maturation of spermatozoa mainly are due to the epididymal secretion and partialy due to the aging process of spermatozoa themselves.[2)4)5)7)8)10)12)24)25)27)32)33)50)63)65)70)72)74)] We can not disregard that the maintenance of the normal epididymal function mentioned above is dependent upon the presence of the testicular hormone.[4)25)35)36)45)] Moreover, it is thought that the spermatozoa are covered with colloidal capsule containing much lipoidal substance during their stay in the epididymes, and that the resistance of the spermatozoa is increased by this matter.[50-52)] Many workers have suported this theory.[6)9)12)32)34)45)48)74)] At any rate, the function of the epididymis are considered as (1) the passage of spermatozoa or the place for the storage of them, (2) the secretion of a small amount of fluid which contributes to the semen and (3) maturation of the spermatozoa.[1)]

As stated above, the spermatozoa show various physiological and morphological changes during the process of important ripening maturation in the epididymes. To obtain the morphological changes of the spermatozoa, especially the morphology and distribution of abnormal ones in various portions of the genital organs the present investigations were undertaken.

I wish to express my gratitude to Prof. S. Nishida for his kind direction.

Materials and Methods

The experimental animals were 20 normal adult male rats belonging to the Wistar strain which has been purebred.

To make the seminal specimens testes and epididymes of the right side of every individuals were taken. The smear of the seminal specimens were made from testes, epididymes (caput, corpus and cauda) and vas deferens. Delafield's hematoxylin was used as stain.

Five hundred spermatozoa were estimated from every seminal specimen. Morphological classification of abnormal spermatozoa was made by the method stated in my previous paper.[37] The characters of the morphology are (1) abnormal headed forms, (2) abnormal necked forms, (3) abnormal middle-pieced forms, (4) abnormal tailed forms, (5) combined abnormal forms and (6) separated spermatozoa (tailless or headless).

Results and Discussion

The percentage of various kinds of the abnormal spermatozoa in the testes, three portions of epididymes and vas deferens are given in Table 1.

Table 1. Kinds and percentages of abnormal spermatozoa in the testes, epididymes, and vas deferens.

Portions	Abnormal headed spermatozoa					Abnormal necked spermatozoa			Abnormal middle-pieced spermatozoa			Abnormal tailed spermatozoa					Combined abnormal spermatozoa	Separated spermatozoa	Sum total
	Deformed	Dwarfish	Malformed	Others	Total	Broken	Others	Total	Coiled or broken	Others	Total	Coiled or broken	Coiled at distal parts of tails	Without posterior tails	Others	Total			
Testes	5.25	0.40	0.50	0.10	6.25	0.67	0	0.67	0.18	0	0.18	0.63	0.25	0.20	0.03	1.10	0.73	10.20	19 13
Caput ep.	4.50	0.34	0.68	0.17	5.64	1.23	0.03	1.26	0.31	0.16	0.47	2.17	0.36	0.05	0.15	2.73	0.96	3.14	14.20
Corpus ep.	3.13	0.18	0.39	0.18	3.88	1.20	0.31	1.51	0.47	0.22	0.69	1.29	0.52	0.02	0.11	1.94	0.99	2.99	12.00
Cauda ep.	3.04	0.24	0.34	0.17	3.79	0.80	0.09	0.89	0.18	0.04	0.22	0.99	0.37	0.08	0.66	1.50	0.64	3.02	10.07
Vas deferens	3.01	0.27	0.29	0.20	3.77	0.67	0.06	0.73	0.17	0.05	0.22	1.27	0.14	0.07	0.07	1.55	0.56	5.63	12.46

As will be seen in Table 1, deformed forms of abnormal headed spermatozoa are the greatest in number. Broken or coiled forms are most numerous among every forms of abnormal necked, middle-pieced and tailed ones. Further observations about the morphology and distribution of abnormal spermatozoa in the normal semen will be fully discussed in another paper which will be issued in near future.[38]

The abnormal spermatozoa given in Table 1 were classified into six kinds, abnormal headed, necked, middle-pieced, tailed, combined and separated forms, and the test of significance of the differences among percentages of various forms was made by the method of "t test." The results are shown in Table 2.

Total percentage of abnormal spermatozoa. As is shown in Table 2, the total percentage of abnormal spermatozoa is the highest in the testes, decreasing as the spermatozoa pass through the epididymes, and is the lowest in the cauda epididymides ; and then in the vas deferens increase in some degree. Both the difference between the percentage in the testes and that in the caput epididymides and that between the percentage in the cauda epididymides and that in the vas deferens are significance respectively. As will be stated later, it is due to a large number of separated cells contained in the testes that the abnormal percentage is high in the testes. The same relation is also found in the vas

Table 2. Kinds and percentages of abnormal spermatozoa

Portions	Abnormal headed spermatozoa	Abnormal necked spermatozoa	Abnormal middle-pieced spermatozoa
Testes	6.25	0.67	0.18
	$0.5<p<0.6$	$0.02<p<0.05$	$0.1<p<0.2$
Caput ep.	5.64	1.26	0.47
	$0.01<p<0.02$	$0.5<p<0.6$	$0.05<p<0.1$
Corpus ep.	3.88	1.51	0.69
		$0.05<p<0.1$	$0.05<p<0.1$
Cauda ep.	3.79	0.89	0.22
		$0.4<p<0.5$	
Vas deferens	3.77	0.73	0.22

deferens. The fact that the percentage of abnormal spermatozoa in the cauda is lower than that in the other parts is of great interest as will be fully discussed later.

Abnormal heads. Numerous abnormal headed forms exist in the testes or in the caput epididymides, the percentage of them following after that of the separated cells. The difference among the percentages of the abnormal headed forms in every parts of genital organ has not noteworthy significance, though the percentage of them in the caput is higher in some degree than that in the corpus.

The morphological observations on the abnormal spermatozoa in the semen taken from the cauda epididymidis and those in the ejaculated semen of mammals have been described by many workers, agreeing in their opinions that the abnormal tailed forms or the tailless ones are much in the semen of the normal animals.[14)-16)28)-30)46)57)58)60)64)67)] On the contrary, however, the abnormal headed forms found in the present investigation on the male rats were much more than the abnormal tailed ones. This fact coincides with my unpublished data obtained from the normal male rats.[42)]

Abnormal necks. The abnormal necked forms in the caput epididymides are more numerous clearly than those in the testes, and those in the corpus epididymides are large number in some degree companing with those in the cauda. Accordingly, it is considered that these abnormal cells are mainly formed in the caput epididymides or in the corpus epididymides, and partialy in the testes.

Abnormal middle-pieces. The abnormal middle-pieced spermatozoa appear in the least percentage of all the kinds of abnormal spermatozoa. A few of these forms also appear in the testes, increasing in percentage from the caput to the corpus of epididymes, and again decreasing in the cauda epididymides. Accordingly, it is considered that these abnormal forms are formed mainly in the caput epididymides and in the corpus epididymides as the abnormal necked ones do so.

in various portions, and the test of differences by "t test".

Abnormal tailed spermatozoa	Combined abnormal spermatozoa	Separated spermatozoa	Total
1.10	0.73	10.20	19.13
p<0.01	0.01<p<0.02	p<0.01	0.02<p<0.05
2.73	0.96	3.14	14.20
0.05<p<0.1			0.1<p<0.2
1.94	0.99	2.99	12.00
0.1<p<0.2	0.2<p<0.3		0.2<p<0.3
1.50	0.64	3.02	10.07
	0.4<p<0.5	p<0.01	0.02<p<0.05
1.55	0.56	5.63	12.46

Abnormal tails. A small number of the abnormal tailed spermatozoa appear in the testes, the percentage of them being the lowest of all portions, but they increase clearly in the caput epididymides, and again decrease in some degree in the corpus and cauda. Accordingly, it is considered that the abnormal tailed spermatozoa are mainly formed in the caput and partially in the testes. The appearance of the abnormal tailed spermatozoa in the normal condition, as has already been stated by many investigators, is most noticeable. It seems to me that a great number of the abnormal tailed ones might appear under the following conditions; (1) scrotal insulation,[47] (2) nutritional obstacles,[11][40][41] (3) protein-free food ration,[38] and (4) oestrin injection.[39] Indeed, as has been already stated in my previous papers,[38][39] the epididymes became degenerated in the latter two cases and a great number of abnormal tailed spermatozoa appeared, due to the functional obstacles of the epididymes. From the results obtained in the present investigation the author wants to advocate that even in the normal condition, the abnormal tailed forms are also formed in the epididymes, especially in the caput.

Combined abnormals. Most of the combined abnormal forms are composed of mainly combination of both the abnormal tailed and the abnormal headed cones. They are more numerous obviously in the caput epididymides than in the testes.

Separated spermatozoa. In the testes, the separated spermatozoa are found in the largest number of six kinds of abnormal cells, and also in largest number among five portions of genital organs. The cells in the testes are much more numerous than those in the caput epididymides. In the three parts of the epididymes (caput, corpus and cauda) a small number of the cells are found, showing low percentage without exception. It is found that the spermatozoa are numerous in vas deferens. This fact is certainly characteristic to the appearance of separated forms.

The reason why the separated spermatozoa are more numerous in the testes

than in the caput epididymides is not clear, but I suppose that since the testicular spermatozoa are younger cells they are easy to be separated at the neck portion by artifact so as to produce the separated ones. On the other hand, as to the fact that a small number of separated spermatozoa is found in the epididymes, I suppose that some of the separated forms appeared in the testes are degenerated and resorpted during their travel from testes to epididymes.

I will state later about the increase of the separated cells in the vas deferens.

Prosperity and decay of the abnormal spermatozoa among the various portions of epididymes. It has been known that the spermatozoa undergo various changes during maturation by the epididymal secretion and attain the best qualities in the cauda, and that the immature spermatozoa which have protoplasmic drops gradually decrease during their travel from testes to epididymes and become the small number in the cauda epididymides. These relations generally agree with the distribution (per cent) of the abnormal spermatosoa in the every portions of the epididymes. That is, the percentage of abnormal forms gradually decreases during their travel from testes to epididymes and becomes the lowest in the cauda.

As a noticeable fact, every kind of abnormal spermatozoa such as the abnormal headed forms (caput>corpus), abnormal necked forms (corpus>cauda), abnormal middle-pieced forms (corpus>cauda), and abnormal tailed forms (caput>corpus) decrease in more or less degree during their travel from the caput to the cauda of the epididymes. As to the reason why the abnormal cells show a small number in the cauda epididymides, notwithstanding they show a large number in the caput, I notice the following relations : First, some of the abnormal cells appearing in the testes or in the caput epididymides might be destroyed and absorped in the corpus epididymides or in the cauda epididymides, though some investigators[61)74)] stated that the destruction or absorption of the abnormal cells normally occur in the vas deferens, second, some of the abnormal ones formed in the proximal end of the ductus epididymidis might be reformed to the normal forms in the distal portion of the epididymis. If the last relation is accepted, two kinds of abnormal spermatozoa, the one is perfect abnormal form and the other imperfect form which having not yet investigated, nevertheless should be found in the caput and in the corpus of epididymis. This will be fully discussed in near future.

Abnormal spermatozoa in the vas deferens. The abnormal spermatozoa in the vas deferens are greater number than those in the cauda epididymides. McKenzie & Phillips ('34)[30)] reported on rams that abnormal spermatozoa in the semen ejaculated during early breeding season or during following periods of breeding inactivity are of mostly tailless or coiled tailed forms. McKenzie & Berliner ('37)[28)] on the ram, Swanson & Herman ('40)[64)] on the bull, also noted

respectively that the first ejaculates during the off season usually contained a higher proportion of abnormal spermatozoa than the later ejaculates during the same season. Stiasny & Generals ('37)[62] found that the percentage of abnormal spermatozoa in the epididymis, vas deferens, and ampulla ductus deferens of bulls are 4.1, 5.2 and 5.3, respectively. Saeki ('48)[57] reported that the percentage of the abnormal spermatozoa in the ampulla ductus deferens of male goat are more numerous than those in the cauda epididymides. Herman & Swanson ('41)[14] and Young ('29)[72] reported respectively that the spermatozoa stored for long time in the cauda epididymidis decreased the fertilizing capacity due to the over-ripening of the spermatozoa. As stated above, it is known that the abnormal spermatozoa increase when the semen were stored for a long time in the vas deferens, and that the abnormal spermatozoa in the vas deferens are more than those in the epididymis. Almost similar results are obtained in the present investigation on albino rats. Namely, as will be seen in Table 2, the percentage of the abnormal spermatozoa are higher in the vas deferens than in cauda epididymidis.

Summary

Morphology and distribution of the abnormal spermatozoa appearing in the five parts of genital organ (testes, caput epididymides, corpus epididymides, cauda epididymides and vas deferens) of 20 normal matured albino rats were investigated. The results are summarized as follows:

1) Six kinds of the abnormal spermatozoa, such as the abnormal headed forms, abnormal necked forms, abnormal middle-pieced forms, abnormal tailed forms, combined abnormal forms and separated forms, were found in the testes, epididymes and vas deferens.

2) Total percentage of the abnormal spermatozoa are the highest in the testes, decreasing from the proximal to distal end of the epididymes, and then in the vas deferens increase in some degree.

3) Both the separated forms and abnormal headed ones are most numerous among various kinds of abnormal spermatozoa.

4) The abnormal necked forms are the most numerous in the corpus and in the caput of epididymes.

5) The abnormal middle-pieced forms are found in the lowest number among the various kinds of the abnormal ones, though some large number of them are found in the corpus epididymides.

6) The abnormal tailed forms are numerous in the caput epididymides, decreasing in the corpus and the cauda epididymides.

7) The combined abnormal forms are numerous in the caput and corpus epididymides.

8) A great number of the separated spermatozoa are observed in the testes, a small number of them in the epididymes and enormously larger number of them in the vas deferens.

9) The percentage of the abnormal fomrs are numerous in both the testes and vas deferens, due to the large number of the separated cells appearing in the both portions.

10) It is supposed that the abnormal spermatozoa are mainly formed in the caupt epididymides and partialy in the testes.

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